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Validation of the synonymy of the teleost blenniid fish species *Salarias phantasticus* Boulenger 1897 and *Salarias anomalus* Regan 1905 with *Ecsenius pulcher* (Murray 1887) based on DNA barcoding and morphology

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Abstract

As currently recognized, *Ecsenius pulcher* includes *Salarias pulcher* (type material has a banded color pattern), *S. anomalus* (non-banded), and *S. phantasticus* (banded). The color patterns are not sex linked, and no other morphological features apparently distinguish the three nominal species. The recent collection of banded and non-banded specimens of *Ecsenius pulcher* from Iran has provided the first tissue samples for genetic analyses. Here we review the taxonomic history of *E. pulcher* and its included synonyms and genetically analyze tissue samples of both color patterns. *Salarias anomalus* is retained as a synonym of *E. pulcher* because DNA barcode data suggest that they represent banded and non-banded color morphs of a single species. Furthermore, the large size of the largest type specimen of *S. anomalus* (herein designated as the lectotype) suggests that it belongs to *E. pulcher.* A single non-banded specimen from Iran is genetically distinct from *E. pulcher* and appears to represent an undescribed species. *Salarias phantasticus* is retained as a synonym of *E. pulcher* because the primary morphological difference between the two nominal species—presence of spots on the dorsal fin in *E. pulcher* and absence of those spots in *S. phantasticus*—is not a valid taxonomic character; rather, the spots represent galls that contain the larval stages of a parasitic crustacean. As males and females of *Ecsenius* species have been confused in the literature, we describe and illustrate the genital regions of both and comment on possible new blenniid synapomorphies that our investigation revealed.

Key words: Blenniidae, taxonomy, Color polymorphism, Oman Sea, Iran, Integrative taxonomy, Parasitic crustacean, Blenniid sex anatomy

Introduction

The Blenniidae comprise 58 genera and 406 species of typically small, marine, benthic fishes (compiled April, 2015, from on-line *Catalog of Fishes*—http://researcharchive.calacademy.org/research/Ichthyology/catalog/fishcatmain.asp). Most species occur on the nearshore substrata of the tropical and temperate marine waters of the Indo-west Pacific. The Indo-west Pacific genus *Ecsenius,* comprising 53 species, is the most speciose. McCulloch (1923:121) described the genus to accommodate his new species, *E. mandibularis,* from Australia. He did not include any other species in the genus. Norman (1943:810), in what can be called the first modern revision of blenniid genera, assigned *Salarias pulcher* Murray1887 and *S. anomalus* Regan 1905 to *Ecsenius*, but did not mention *Salarias phantasticus* Boulenger 1897, which would have been easily assigned to *Ecsenius.* The omission is surprising, as the type series of all three species are housed in the BMNH collection, where Norman was working at the time.

Chapman & Schultz (1952) first brought together all blenniid species (including several new ones) then assignable to *Ecsenius.* Except for *E. mandibularis,* all the previously described species they included had

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originally been assigned to *Salarias*. They synonymized *S. phantasticus* and *S. anomalus* under *S. pulcher,* believing that the banded pattern present in the two syntypes of *S. phantasticus* and two syntypes of *S. pulcher* was restricted to males, whereas the non-banded pattern present in all of the 16 syntypes in the single lot of *S. anomalus* they examined was restricted to females of the same species. They noted the presence of dark spots in the dorsal fins of two "male" specimens, but they did not indicate which type specimens (*S. pulcher* or *S. phantasticus*) exhibited those spots.

Springer (1971) next revised *Ecsenius*. He examined the syntypes of *S. pulcher* and *S. phantasticus* and the same lot of 16 syntypes of *S. anomalus* and accepted Chapman and Schultz's synonymy of the latter two species under *E. pulcher,* but he noted that the two color patterns are not sex linked. The two banded syntypes of *E. pulcher* comprise one male and one female (versus two males as indicated by Chapman & Schultz 1952), and the single lot of 16 non-banded syntypes of *E. anomalus* comprise seven males and nine females (vs. all females as indicated by Chapman & Schultz 1952). Except for color pattern, there did not appear to be any other morphological differences between the banded and non-banded specimens attributed to *E. pulcher,* and Springer (1988), in an updated revision of *Ecsenius*, continued this synonymy. We discuss below two additional lots of *S. anomalus* that Springer (1971, 1988) overlooked.

The recent collection of fresh material from Iran allowed the first genetic analysis of the two color morphs (Estekani 2014). A comparison of the mtDNA barcode (cytochrome *c* oxidase subunit I—COI) of one specimen of each of the two color patterns from Chabahar Bay, Iran, indicated a 4% difference between the banded and non-banded patterns (Estekani 2014). It was initially believed that this validated removal of the uniformly colored *S. anomalus* from the synonymy of *S. pulcher*. With only one specimen of each color pattern, however, it was not possible to distinguish intra- and interspecific genetic variation.

Herein we further review the taxonomic history of the involved species; discuss the results of the genetic analyses; make taxonomic decisions regarding the status of *Salarias anomalus* and *S. phantasticus* based on the genetic data and subsequent morphological investigation (including the dark spots observed in the dorsal fin of *E. pulcher* by Chapman & Schultz, which we further investigate); and describe the external differences in the genital region of male and female *Ecsenius,* confusion about which has historically led to errors in sex determination.

Material and methods

Specimen collection. Banded and non-banded specimens of *E. pulcher* were collected from Chabahar Bay, Oman Sea, Iran, by SCUBA diving using a hand net. Specimens for DNA Barcoding were stored in a -20C freezer or 95% ethanol.

DNA extraction, amplification, and sequencing. For the six specimens genetically analyzed at the Smithsonian's National Museum of Natural History, DNA extraction, PCR, sequencing COI, and editing sequences were performed as outlined by Weigt *et al.* (2012a). For specimens genetically analyzed at Chabahar Maritime University, total genomic DNA was extracted from muscle tissue (without skin) using the CTAB protocol (Thollesson, 2000). The quality and quantity of extracted DNA was detected on 1% agarose gel electrophoresis and ethidium bromide staining. Fifteen ng/µl of the extracted DNA was used as a template in polymerase chain reaction (PCR) amplification to amplify approximate 700bp of COI. In the PCR reaction, the forward primer was LCo11490 (5′-GGTCAACAAATCATAAAGATATTGG -3′) and the reverse primer was CO1HCO2198(5′-AAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.* 1994; Carreras-Carbonell *et al.* 2005). Amplifications were carried out in 50-µL total volume with NH4 plus, PCR buffer, 3 mM MgCl2, 200 mM dNTPs, 10 pM of each primer, 1 U of BioTaq DNA polymerase (Bioline, UK), and 10 ng of DNA template (Thollesson 2000). PCR was performed under the following conditions: in a Primus 96 (MWG Biotech), and cycle parameters consisted of a first denaturing step at 94 °C for 2min, followed by 35 cycles of 1min at 94 °C, 1min at the optimal annealing temperature 40°C and 1min at 72 °C, and a final extension at 72 °C for 7min. (Carreras-Carbonell *et al.* 2005). PCR products were cleaned up by montage PCR clean-up columns (Millipore, USA) (Attaran-fariman & Bolch 2013) and then sequenced according to manufacturer protocols.

Genetic analyses. For comparative purposes, COI sequences for additional *Ecsenius* species were downloaded from GenBank http://www.ncbi.nlm.nih.gov/genbank/) and BOLD (http://www.boldsystems.org/). Sequences were aligned using ClustalX version 1.83 (Thompson *et al.* 1997). A neighbor-joining tree (Saitou & Nei 1987) was

generated using PAUP*4.1 (Swofford 2002) for the purpose of tabulating Kimura two-parameter distances (Kimura 1980). The neighbor-joining analysis reveals genetic distances in COI among individuals and clusters them into genetically distinct lineages, which, in teleost fishes, correspond well with species (e.g. Baldwin & Weigt 2012, Weigt *et al.* 2012b). Interspecific phylogenetic relationships were hypothesized based on maximum parsimony analysis of the COI sequences using heuristic searches in PAUP*4.1 (Swofford 2002). Characters were equally weighted and left unordered. The resulting equally parsimonious trees were summarized using the strict consensus method, and nodal support was estimated from 1,000 replicates of the bootstrap, utilizing random addition sequence and TBR branch swapping (Swofford 2002). The outgroup for both analyses was *Cirripectes quagga,* which was selected based on its position as a basal member of the sister group of *Ecsenius* in the phylogeny of Lin & Hastings (2013). *Istiblennius edentulus,* a less basal member of this sister group, was also included in the analyses.

Nomenclature and accession numbers. GenSeq nomenclature (Chakrabarty *et al.* 2013) and GenBank accession numbers for DNA sequences derived in this study are presented along with museum catalog numbers for voucher specimens in the Appendix. GenBank numbers for comparative material included in the genetic analysis are as follows: *Cirripectes quagga* (JQ431647 and JQ431648), *Istiblennius edentulus* (JF493684 and JQ350065), *Ecsenius opsifrontalis* (HQ168559), *E. bicolor* (FJ583381 and FJ583382), *E. midas* (JQ349942-JQ349944 and JF493406), and *E. nalolo* (JQ349946, JQ349948, KF929830).

Morphological investigation. Body measurements were made with needle-point dial calipers months to years after preservation. Counts of median fin rays and vertebrae were made from either digital radiographs or x-ray film. Images of the spots in the dorsal fin and of the genital regions of one male and one female were taken with a Zeiss Axiocam attached to a Zeiss Stereo Discovery V12. The final images are composite images prepared with the Zeiss AxioVision software to increase the depth of field.

Results

Further discussion of the taxonomic history of the involved species

Salarias pulcher.—Murray (1887:47) described *Salarias pulcher* from Kurrachee, Manora [now Gulf of Oman, Pakistan] based on two syntypes, male, 44.8 mm SL, and female, 44.5 mm SL, BMNH 1887.9.22.59–60. In his description he indicated the body color pattern as "Anterior two-thirds of the body chocolate brown; posterior third golden yellow, with 5–7 vertical dark bars." He also noted that the dorsal fin of one specimen [male] contained a series of six dark spots and the dorsal fin of the other specimen [female] contained only two dark spots. He did not differentiate his new species from any other species.

Springer (1988:32) designated the male syntype (Fig. 1A) as lectotype, and assigned it catalog number BMNH 1887.9.22.59. It can be readily distinguished from the female paralectotype by the number of spots in the dorsal fin and in having 6 dark bands posteriorly on the left side and 5 dark bands on the right side of the body. The female paralectotype (which was assigned catalog number BMNH 1887.9.22.60) has 5 dark bands on the left side, with a faintly dusky band between the first two dark bands, another between the fourth and fifth dark bands, and questionably another posterior to the fifth dark band; there are 6 dark bands on the right side of the body with a very faint band between the first two dark bands and another between the fourth and fifth dark bands.

Salarias phantasticus.—Boulenger (1897:422) described *Salarias phantasticus* from the Mekran Coast of Persia [now Gulf of Oman coast of Iran] based on two male syntypes, 54.5 and 58.7 mm SL, BMNH 1897.9.22.20-21. He described the color pattern as "Head and anterior half of body dark brown; posterior half of body orange, with eight vertical black bars." He did not indicate the presence of dark spots in the dorsal fin of either specimen, and we confirm that there are none. He did not differentiate his new species from any other species, but he possibly considered the absence of dorsal-fin spots in his specimens as indicative of a species distinct from the otherwise very similar *S. pulcher* (compare Figs 1A and 1B**)**. To clearly distinguish the lectotype from the paralectotype and firmly establish the taxonomy of the species, we here designate the longer specimen (Fig. 1B) as lectotype, catalog number BMNH 1897.9.22.21. It has eight dark body bands on its left side and seven on the right side. The smaller specimen (paralectotype), BMNH 1897.9.22.20, has seven dark bands on both the left and right sides of the body.

Salarias anomalus.—Regan (1905:327) described *Salarias anomalus* from "several specimens [syntypes]

from the Persian Gulf and Mekran Coast." He also indicated later in the text that the species [= specimens?] was from the Gulf of Oman, Mekran coast, and, confusingly, Karachi [Pakistan]. Springer (1971, 1988), who twice revised *Ecsenius,* only indicated the 16 Persian Gulf specimens in BMNH 1900.5.9.47–56 as syntypes of *S. anomalus,* but did not designate a lectotype. Smith-Vaniz & Springer (1971), without comment, noted that the syntypes of *S. anomalus* comprise two lots: BMNH 1900.5.9.47–56 and 1899.5.8.94–95 (2 non-banded females, 28.5 and 34.3 mm SL from Jask, Mekran coast). We here note that BMNH 1898.6.29.163 (non-banded male 57.4 mm SL), collected by Townsend from Hinderabi Island, Persian Gulf, is also a syntype, making a total of 19 syntypes of *S. anomalus*. We know of no other specimens collected by Townsend that might possibly be syntypes of *S. anomalus,* despite the fact that Regan included *S. anomalus* in his list of fishes from the Mekran coast and Karachi. Regan followed this list with the following statement: "Karachi falls within the province treated of Day's 'Fishes of India', and the additions, except in the case of the new species described above, are unimportant." This explains why there is no syntype of *S. anomalus* from Karachi.

Regan did not compare his new species with any other species, but probably considered that it differed from *S. pulcher* and *S. phantasticus* in lacking bands (Fig. 1C). Regan (1905: pl. B, fig. 4) illustrated one specimen that he thought (correctly) might be a male, but we cannot assign it with certainty to any one of the syntypes. The extremely elongated dorsal-fin spines shown in the figure occur only in males (the spines may be elongated in females, but never as extremely as in Regan's figure).

FIGURE 1. Lectotypes of the nominal species of *Ecsenius pulcher*. A, *Salarias pulcher* Murray, BMNH 1887.9.22.59, male, 44.8 mm SL; B, *Salarias phantasticus* Boulenger, BMNH 1897.9.22.21, male, 58.7 mm SL; C, *Salarias anomalus* Regan, BMNH 1900.5.9.47, male, 58.4 mm SL.

FIGURE 2. A, banded and B, non-banded specimens of *Ecsenius pulcher* analyzed genetically (SMF 35774, 47.0 mm SL and SMF 35773, 84.0 mm SL, respectively); C, non-banded specimen of *Ecsenius* sp. that is genetically distinct from *E. pulcher* (52.0 mm SL, no cataloged voucher specimen).

Genetic analyses. Examples of banded and non-banded specimens recently acquired for the genetic analysis are shown in Figure 2. Figure 3 shows the results of the maximum parsimony analysis, indicating that all of the banded and non-banded ("uniform") specimens form a monophyletic lineage, with respect to other species in the analysis, with 100% bootstrap support. However, a monophyletic group comprising all of the banded and most of the non-banded specimens (*"E. pulcher"* in Figure 3) is distinct from the single non-banded specimen of Estakani's (2014) original analysis ("*Escenius* sp." in Figure 3). The *E. pulcher* lineage is 4.0–5.4% (average 5.0) divergent in COI from *Ecsenius* sp. (Table 1), which is much higher than typical intraspecific variation in COI in marine fishes (e.g., Ward *et al.* 2005, Rock *et al.* 2008, Ward *et al.* 2008, Zemlak *et al.* 2009, Tornabene *et al.* 2010, Baldwin *et al.* 2011, Baldwin & Weigt 2012, Zhang & Hanner 2012). *Ecsenius pulcher* and *Ecsenius* sp. differ from other *Ecsenius* species included in the analysis by 17.4–24.6%. Although not tabulated in Table 1, the genetic distance between the two *Istiblennius edentulus* sequences acquired from GenBank (one from South Africa, one from Madagascar) differ from one another by 19.8%; as this is very high divergence, further investigation is needed. Possibly *I. edentulus* includes a cryptic species, or one of the specimens from which the sequences were derived was misidentified.

TABLE 1. Average (and range) Kimura two-parameter distance summary based on cytochrome *c* oxidase I (COI) sequences of *Ecsenius* species represented in the cladogram in Figure 3. Intraspecific averages are shown in bold.

	E. bicolor N = 2	*E. midas* N = 4	*Ecsenius* sp. N = 1	*E. pulcher* N = 7	*E. nalolo* N = 3	*E. opsifrontalis* N = 1
E. bicolor	0					
E. midas	17.4–18.2 (17.7)	0.5–1.1 **(0.7)**				
Ecsenius sp.	21.8	23.6–24.1 (23.8)	na			
E. pulcher	20.1–20.7 (20.5)	22.9–24.6 (23.8)	4.0–5.4 **(5.0)**	0.8–1.4 **(1.1)**		
E. nalolo	19.4–20.0 (19.7)	20.9–24.0 (22.1)	17.6–24.7 (19.6)	0.8–1.4 (1.1)	0.4–0.6 **(0.5)**	
E. opsifrontalis	20.1	22.5–22.9 (22.7)	19.4	19.6–20.8 (20.3)	19.1–19.4 (19.2)	na

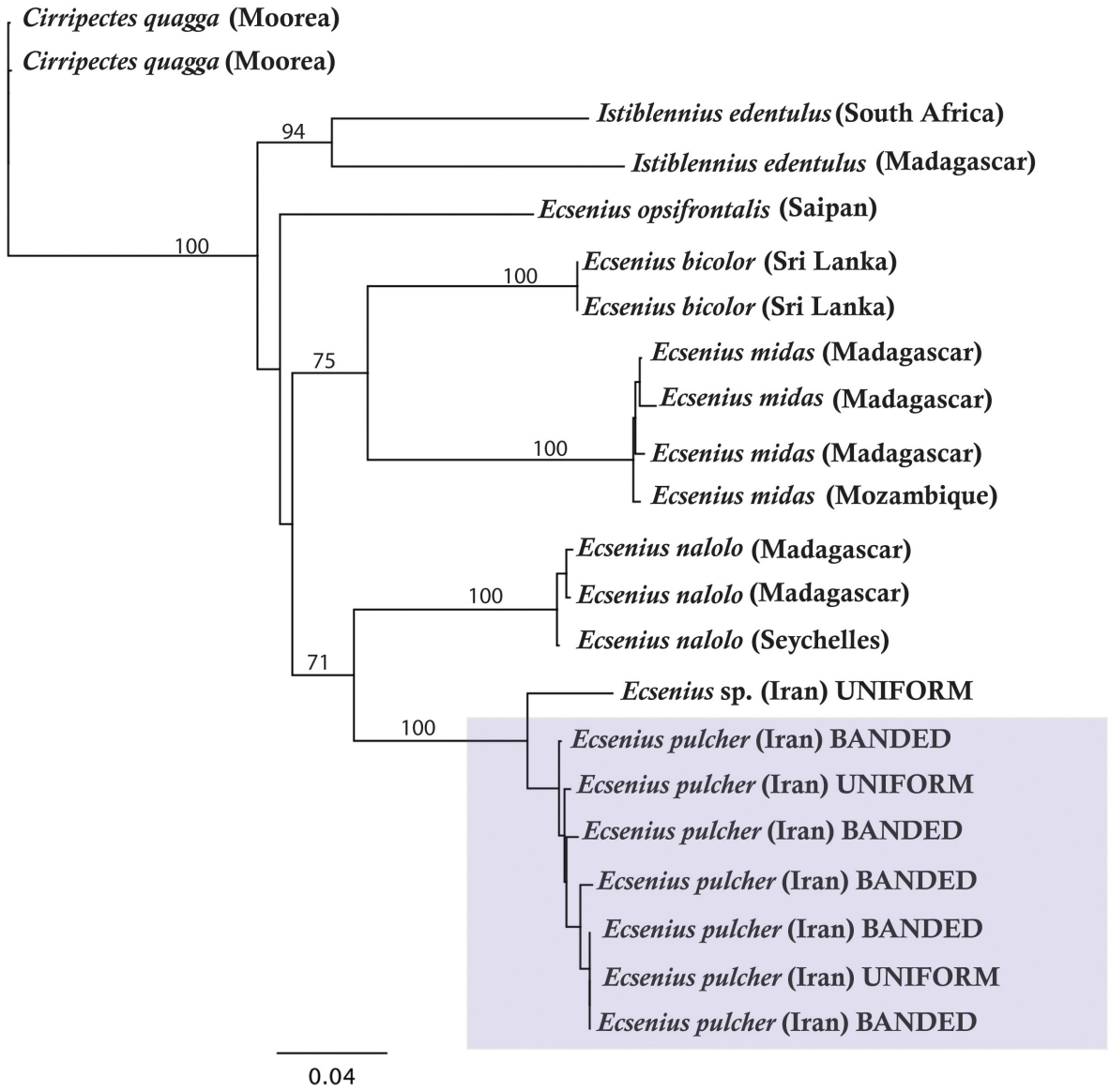

FIGURE 3. The strict consensus of a maximum parsimony analysis of the COI region of eight individuals of *Ecsenius* from Iranian waters and related taxa. The outgroup for the analysis was *Cirripectes quagga.* Numbers above branches represent bootstrap support values > 50.

Taxonomic Status of *Salarias anomalus.* Previous morphological analyses provided insufficient evidence to recognize *Salarias anomalus* as distinct from *E. pulcher,* and the two differ primarily in color pattern (non-banded in *S. anomalus,* banded in *E. pulcher*). However, our data suggest that non-banded (uniform) color patterns comprise two genetically distinct lineages, one (*E. pulcher* in Fig. 3) that groups with the banded *E. pulcher,* and a single uniform specimen (*Ecsenius* sp. in Fig. 3) from Estakani's (2014) original analysis that constitutes a separate genetic lineage. Ongoing investigation suggests that *Ecsenius* sp. represents an undescribed species that will form the basis for another study. This specimen is con-specific with specimens showing a different color pattern, which have a more southern distribution (south to Socotra Island) than *E. pulcher* (south to about 22°25' N on the northeasternmost coast of Oman). Although numbers of fin rays, vertebrae, and teeth do not appear to distinguish the two species, the new species is smaller (51 mm SL or smaller) than *E. pulcher* (to 81 mm SL). Because the 19 syntypes of *S. anomalus* range in size from 29.7–58.4 mm SL—the longest thus being considerably longer than 51 mm SL—we conclude that *S. anomalus* is indeed a synonym of *E. pulcher.* To assure the distinction that a non-banded *anomalus*-like syntype is identifiable as *S. pulcher,* we here designate the longest syntype, a 58.4-mm SL male in BMNH 1900.5.9.47–56, as lectotype (Fig. 1C). It is assigned the catalog number BMNM 1900.5.9.47. The remaining 15 specimens in that series are assigned BMNH 1900.5.9.48–56 and become paralectotypes. The two syntypes in BMNH 1899.5.8.94–95 and the single syntype in 1899.5.8.94 now become paralectotypes.

Taxonomic status of *Salarias phantasticus* and the nature of the dorsal-fin spots in *Ecsenius pulcher.* *Salarias phantasticus* has a similar banded pattern as the type material of *E. pulcher,* and there is little reason to question the synonymy with *E. pulcher* except for the absence of spots on the dorsal fin in *S. phantasticus* (Fig. 1B). We investigated the dorsal-fin spots like those present in the syntypes of *S. pulcher* (Fig. 1A) and found that they are not limited to banded specimens but may also be present in the dorsal fins of non-banded specimens of *E. pulcher*. The spots, however, appear to be present only in some specimens from outside the Persian Gulf. When present, they are primarily restricted to the spinous portion of the dorsal fin and some of the more anterior segmented rays. Chapman & Schultz (1952:517) were first to notice something peculiar about the dark dorsal-fin spots of the syntypes of *S. pulcher*, "…the last two [dorsal-fin] spots [of the male] thickened and involving the spines." Although VGS twice examined the syntypes at BMNH, he recorded no data on the obvious peculiar nature of the spots, possibly deferring to Chapman & Schultz's comment.

We re-examined radiographs made in the late 1980's of *E. pulcher* specimens from the vicinity of Sur, Oman, on the southeastern-most coast of the Gulf of Oman, deposited in the Royal Ontario Museum (ROM). During the re-examination, VGS noticed that the dorsal fins of some specimens of both banded and non-banded color patterns appeared to show indications of the dorsal-fin spots. Because color pattern does not show up on radiographs, the thought immediately arose that these spots were anomalies, and a loan to USNM of specimens exhibiting the spots was requested from ROM. Two of the seven lots of ROM specimens that had dorsal-fin spots were sent. One lot contained a specimen of uniform color pattern (Fig. 4B), female, 52.8 mm SL, with 6 spots, ranging from very weak to well developed in the dorsal fin. The other lot contained one banded color pattern, female (Fig. 4A) 52.6 mm SL, with 9 mostly well-developed spots, and 2 uniform females, one (56.7 mm SL) with 6 mostly well-developed spots, and the other (53.2 mm SL) with no spots in the dorsal fin. Each spot was associated with a capsule (Fig. 4C), one of which was partially opened and found to include a tiny parasitic crustacean (Fig. 5). The capsules are termed galls by specialists who study parasitic crustaceans. The dark spots are external discolorations associated with the infection and possibly serve to protect the parasite, which would otherwise be apparent through the transparent membrane of the dorsal fin.

At the suggestion of Chad Walter (USNM), we contacted, Danny Tang (Orange County Sanitation District, Fountain Valley, CA), who has specialized knowledge of parasitic crustaceans, and sent him copies of the photographs of the parasite. He identified it as a late-stage developing male specimen, probably a new species, of the pandarid genus *Amaterasia* Izawa. He was reluctant to describe the species based on a non-adult specimen. He informed us that there are only two described species of *Amaterasia*: *A. amanoiwatoi* Izawa, known only from the balistid *Xanthichthys lineopuncatus* (Hollard) taken off Clarion Island in the eastern Pacific, and *A. lewisi* Tang, Benz & Nagasawa 2012, reported variously from acanthurids, a diodontid, zanclid, labrid, scarid, taken at either Midway Island or Oahu, HI, or from the Honolulu Aquarium, presumably from Hawaii. In summary, the presence of "spots" in the dorsal fin is the result of parasitic infection, not natural pigmentation, and it provides no taxonomic information. We retain *S. phantasticus* as a synonym of *E. pulcher.*

FIGURE 4. Examples of female banded and non-banded specimens of *Ecsenius pulcher* from near the southwesternmost coast of the Gulf of Oman having dorsal-fin galls harboring the parasitic copepod *Amaterasia* sp. A, (ROM 40186); B (ROM 98136); C, close-up photo of dorsal fin of A. See Figure 1A for an example of a male specimen with galls.

Remarks on the external differences in the urogenital region of *Ecsenius* specimens. In all blenniid males the first two anal-fin spines are well developed and although smaller than the first segmented ray, are distinguishable externally from the ray. The urogenital papilla is usually a tiny simple tube situated immediately anterior to the first spine and just posterior to the anus. It usually incorporates a single canal through which both sperm and urine pass. Eggert (1931) described and illustrated the male blenniid papilla of many blenniid species using mostly currently invalid generic names. Longley & Hildebrand (1941:271, pl. 31, fig. 2) described and illustrated the male papilla of *Ophioblennius truncatus* (Poey 1861), as *Rupiscartes atlanticus* (non *Salarias atlanticus* Valenciennes 1836), as follows: "The sperm ducts open separately in a pair of elevated points at the outer ventral margin on either side of the flattened urogenital papilla." Presumably, the ducts also serve as the excretory openings for urine. We examined the external structure of the papilla of a male specimen of *Ophioblennius* sp. that agrees with Longley & Hildebrand's description. Males of *Ecsenius* (e.g., SMF 35783, *E. frontalis* [*albicaudatus* form], 51.1 mm SL, Yemen - Fig. 6, top), have little if any modification of the papilla or, apparently, the epidermis covering the anal-fin spines or segmented rays, but in various other blenniid genera, the epidermis surrounding the anal-fin spines and the segmented rays of mature males is modified. The modifications take many forms ranging from fleshy rugose knobs enveloping all or just the tips of the spines (e.g., Longley & Hildebrand [1941: pl. 31, fig. 1 *Hypleurochilus*, fig. 2, *Ophioblennius*]) to spatulate or trowel-like folds enveloping the spines and segmented rays (e.g., Springer 1967: pl. 24f, *Entomacrodus nigricans*). These epidermal

modifications have long been known to be glandular and the source of pheromones (Barata & Gonçalves 2009, and references therein).

The external urogenital region of mature blenniid females (Fig. 6 middle, bottom) is quite different from that of males and females of most other fishes, although in sub-adult blenniid females less than about 25 mm the differences may be incompletely manifested or otherwise difficult to distinguish. Many authors have mentioned and/or illustrated the female urogenital region of blenniids. Notable among these are Eggert (1931), Longley & Hildebrand (1941), Tomiyama (1951) and, particularly, Krejsa (1960: fig. 5a).

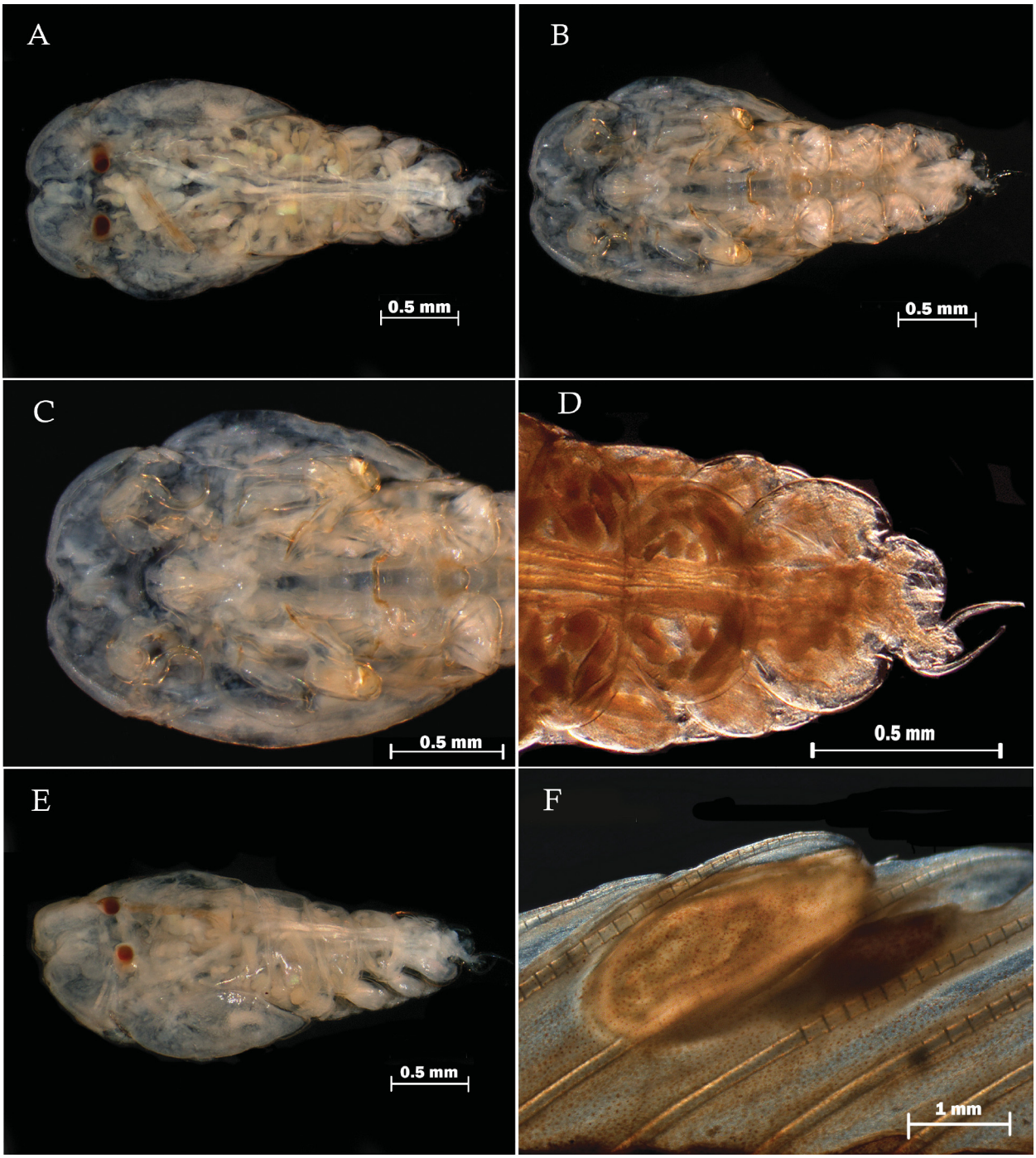

FIGURE 5. *Amaterasia* sp. extracted from gall on dorsal fin of *Ecsenius pulcher*, ROM 40286 (see also Figure 4A). A, dorsal view; B, ventral view; C, close-up of head region, ventral view; D, close-up of tail region, lighting adjusted to highlight furcae, ventral view; E, dorsolateral view; gall from which specimen was extracted with less-developed gall just posterior to it.

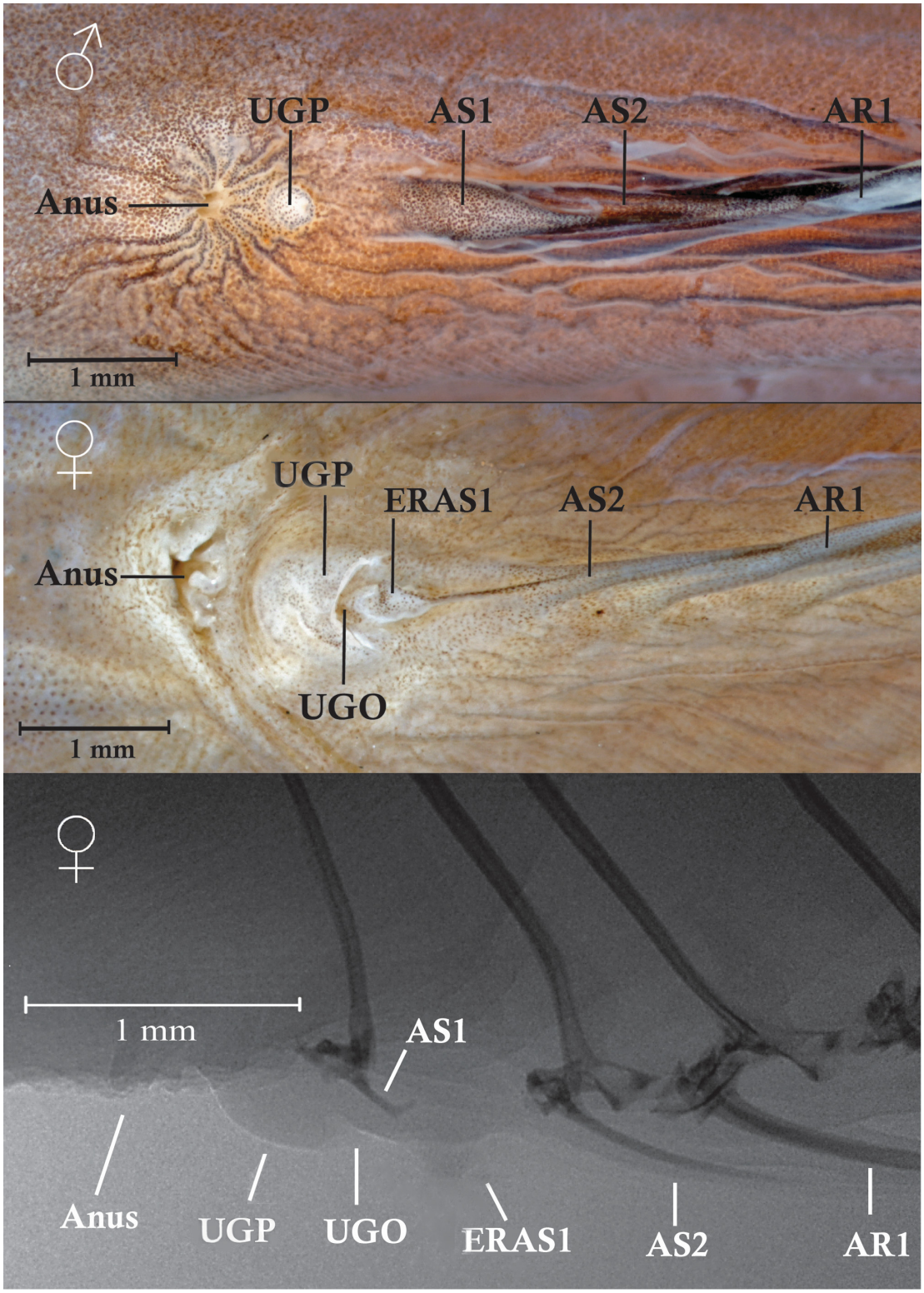

FIGURE 6. Upper, ventral view of external appearance of urogenital area of male *Ecsenius frontalis* (*albicaudatus* form), 51.1 mm SL, SMF 35783. Middle and lower, female *E. pulcher*, 53.2 mm SL, ROM 40185; middle, ventral view of external appearance; lower, reversed radiographic lateral view of urogenital area. AR1—first segmented anal-fin ray; AS1—first anal-fin spine; AS2—second anal-fin spine; ERAS1—epidermal remnant of AS1; UGO—urogenital opening; UGP—modified urogenital papilla (see discussion in text).

Our description and illustrations are based mostly on an *E. pulcher*, female, 53.2 mm SL (ROM 40185). Anal opening relatively large, followed a little posteriorly by a moderately broad, low, mound-like area, representing a

modified urogenital papilla, which forms a ventral anteriorly curving hooded opening leading to the terminal openings of the oviducts and ureters (Eggert, 1931: 413 and fig. 54). The mound-like area continues mid-posteriorly as a reduced and solely epidermal remnant that primitively envelops the first anal-fin spine and is continuous posteriorly with the epidermal sheathing of the second anal-fin spine, but is completely fleshy. The distal epidermal tip of the remnant frequently appears as a tiny non-patent papilla, which originally led us to believe that it was the urogenital papilla. Embedded in the mound-like area just dorsal to the papilla-like continuation with the second anal-fin spine is the remnant of the first anal-fin spine and its pterygiophore (Fig. 6C), which have been displaced well anteriorly to second anal-fin spine and its pterygiophore, as compared with the disposition of these elements in males. Krejsa (1960) indicated that the tip of the first anal-fin spine was present near the urogenital opening in *Blenniolus* (= *Hypsoblennius* Gill), a blenniinine genus, less specialized than *Ecsenius*, a salariinine.

Our brief survey of blennioid genera suggests that the nature of the female genital area described above is probably a blenniid synapomorphy. The various manifestations of the modifications of the skin enveloping the anal-fin spines and often some of the associated segmented rays of males apparently does not involve males of all blenniid genera or species, but may also be a blenniid synapomorphy. Further investigation of the external or internal sexual manifestations of blenniids is beyond the purposes of the present study.

Discussion and conclusions

DNA barcoding (e.g., Hebert *et al.* 2003) has proved useful in illuminating cryptic species diversity and in resolving complex taxonomic issues, including in groups of small tropical shorefishes such as gobies and blennies (e.g., Tornabene *et al.* 2010, Baldwin *et al.* 2009, 2011, Baldwin & Robertson 2015; Victor 2013). In this study DNA barcoding provided a test of the limits of *Ecsenius pulcher,* which currently includes both banded and non-banded color morphs previously assigned to *Salarias pulcher, S. anomalus,* and *S. phantasticus.* The DNA data and subsequent morphological investigation support the synonymies of *S. anomalus* and *S. phantasticus* with *E. pulcher* and reveal the existence of what appears to be a cryptic species that will be the subject of a future paper. Detailed morphological investigation of what appeared to be pigment spots on the dorsal fins of some *E. pulcher* specimens revealed that they are actually galls containing what may represent an undescribed species of parasitic crustacean. Integrative taxonomic studies such as the one conducted here that combine molecular and morphological analyses will continue to improve our understanding of blenniid shorefish diversity. Additional collecting efforts are essential (e.g., Rocha *et al.* 2014), especially in poorly studies areas such as some portions of the Middle East.

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APPENDIX. Links between DNA voucher specimens, color pattern, GenBank accession numbers, and cytochrome c oxidase subunit I (COI) sequences of *Escenius* specimens analyzed genetically.

Species/Catalog Number/Color Pattern	GenBank No.	GenSeq Designation
Escenius pulcher, Banded (voucher at Chabahar Maritime University dried)	KJ411424	Genseq-4 COI
Escenius sp., Uniform (voucher at Chabahar Maritime University dried)	KJ411425	Genseq-4 COI
Ecsenius pulcher, Uniform (5), SMF 35773	KU140948	Genseq-4 COI
Ecsenius pulcher, Banded (1), SMF 35774	KU140951	Genseq-4 COI
Ecsenius pulcher, Banded (2), SMF 35774	KU140952	Genseq-4 COI
Ecsenius pulcher, Banded (3), SMF 35774	KU140947	Genseq-4 COI
Ecsenius pulcher, Uniform (6), SMF 35773	KU140950	Genseq-4 COI
Ecsenius pulcher, Banded (4), SMF 35774	KU140949	Genseq-4 COI